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REVIEW

Polyamines and Their Role in Virus Infection

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SUMMARY Polyamines are small, abundant, aliphatic molecules present in all mammalian cells. Within the context of the cell, they play a myriad of roles, from modulating nucleic acid conformation to promoting cellular proliferation and signaling. In addition, polyamines have emerged as important molecules in virus-host interactions. Many viruses have been shown to require polyamines for one or more aspects of their replication cycle, including DNA and RNA polymerization, nucleic acid packaging, and protein synthesis. Understanding the role of polyamines has become easier with the application of small-molecule inhibitors of polyamine synthesis and the use of interferon-induced regulators of polyamines. Here we review the diverse mechanisms in which viruses require polyamines and investigate blocking polyamine synthesis as a potential broad-spectrum antiviral approach.

KEYWORDS DNA virus, RNA virus, eIF5A, polyamines

INTRODUCTION

For all viruses, the ability to coopt the host cell's resources for their own replication is essential, as viral genomes do not encode protein synthesis machinery, which is necessary for productive infection. Nucleic acids, amino acids, translational machinery, and membranes are all host components commonly appropriated from the infected cell, but the list can extend to signaling proteins and transcription factors as well. Understanding how viruses use host cell resources provides insight into how viruses replicate and, importantly, how these processes could be disrupted to block viral infection. Several antiviral strategies that exploit the viral dependence on host factors have emerged. These range from the use of nucleoside analogs which will block viral

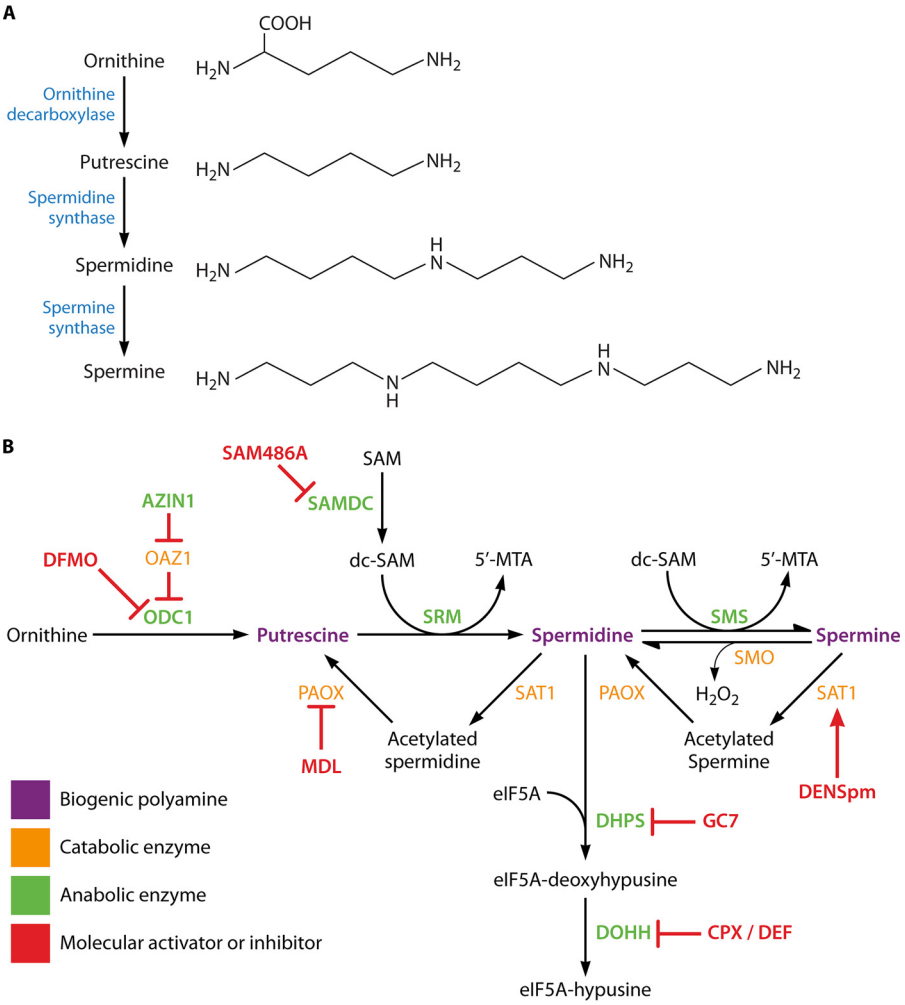

FIG 1 The mammalian biogenic polyamines and metabolic pathways. The polyamines putrescine, spermidine, and spermine (A) are synthesized from the ornithine precursor via a series of enzymatic reactions (B) that elongate the structure of the polyamine and add amino groups. Amino groups are protonated at physiological pH and comprise the aliphatic properties of the polyamines. The biogenic polyamines are highlighted in purple. Anabolic enzymes that promote the synthesis of polyamines are displayed in green, while catabolic enzymes are displayed in orange. Pharmaceuticals targeting the polyamine pathway are highlighted in red. AZIN1, antizyme inhibitor; MTA, 5′-methylthioadenosine; DHPS, deoxyhypusine synthase; SMO, spermine oxidase.

replication due to viral dependence on host-derived nucleotides (e.g., ribavirin for hepatitis C virus) to the use of entry receptor blockers that interfere with the ability of viruses to get into cells (e.g., maraviroc for HIV) (1).

The success of these approaches has led to the search for additional host factors that could be drug targeted to limit virus replication. Among many promising potential host targets that have been described to be important for virus replication, recent studies have revived interest in the role of polyamines in virus replication. Polyamines have now been suggested to have a role in the replication of viruses across all known viral replication strategies and most viral families. This has led to the investigation of inhibitors of polyamine synthesis as inhibitors of many different viruses. Targeting the polyamine biosynthetic pathway may hold promise for the development of broad-spectrum antivirals.

POLYAMINES

What Are Polyamines?

Polyamines are abundant molecules consisting of flexible carbon chains with amino groups that are positively charged at neutral pH. In eukaryotes, there are three biogenic

molecules considered to be polyamines (Fig. 1A), and they are all created through a single synthetic pathway. Bacteria and archaea have a more diverse repertoire of polyamines, including spermidine, homospermidine, norspermidine, putrescine, cadaverine, and 1,3-diaminopropane in bacteria and agmatine, spermidine, homospermidine, norspermidine, and norspermine in archaea. The variety of polyamines present in each of these kingdoms varies among the different organisms (2). The core polyamine synthesis pathway present in mammals is summarized in Fig. 1B. Within the cell, arginine is converted to ornithine, which is converted into the polyamine putrescine via the action of ornithine decarboxylase 1 (ODC1). Putrescine is converted into spermidine via the action of spermidine synthase (SRM); spermidine is converted into spermine via spermine synthase (SMS). Spermine can further be catabolized back to spermidine and putrescine via the action of spermidine/spermine acetyltransferase 1 (SAT1) and polyamine oxidase (PAOX). The cell exerts significant amounts of energy in maintaining polyamine homeostasis through synthesis, degradation, import, and export, highlighting the importance of this pathway to the cell. Polyamines frequently regulate the enzymes involved in their own metabolism, providing a tightly controlled feedback mechanism. For example, ODC1 turnover is regulated by ODC1 antizyme (OAZ1) (3, 4). Translation of OAZ1 is regulated by a frameshifting mechanism that is polyamine dependent (5). ODC1 translation can also be cap dependent or internal ribosome entry site (IRES) dependent at different stages of the cell cycle, providing differential regulation of this enzyme (6). Polyamine levels also affect the translation and activity of *S*-adenosylmethionine decarboxylase (SAMDC), a critical enzyme in the production of spermidine and spermine from putrescine, through the translation of an upstream open reading frame (ORF) (7–10).

Polyamines in Cellular Processes

Putrescine, spermidine, and spermine are found in all mammalian cells, though at various concentrations in different organisms (2). Within the context of a normal healthy cell, polyamines are involved in diverse cellular processes such as protein synthesis, RNA folding and bending, membrane interactions, protein-RNA interactions, DNA structure, and gene expression (Fig. 2) (reviewed in references 11–14). Polyamines bind both RNA and DNA, altering the conformation and function of nucleic acids. Polyamines alter DNA structure by facilitating the conformational transition from the B form to the Z form (15) or by bending DNA (16–18). Furthermore, up to 80% of polyamines in the cell are directly associated with RNA (11), and spermine has also been implicated in the stabilization of tRNA structure (19, 20).

Spermidine further serves as a substrate molecule for the enzyme deoxyhypusine synthase, which acts to posttranslationally generate the unique amino acid hypusine by converting a lysine at amino acid 50 in eukaryotic initiation factor 5A (eIF5A), a cellular translation factor. Hypusination of eIF5A at amino acid 50 is accomplished through a two-enzyme cascade, summarized in Fig. 1B. First, deoxyhypusine synthase (DHS) transfers an aminobutyl moiety from spermidine to the lysine residue on eIF5A (Fig. 1B). The deoxyhypusine residue is then hydroxylated by deoxyhypusine hydroxylase (DOHH) to form the hypusine residue (21). Importantly, eIF5A is the only known protein in the cell that contains hypusine, and hypusination is critical for its eIF5A function (22). Within the cell, hypusinated eIF5A has been suggested to play many roles. In addition to its original identification as a stimulator of dipeptide synthesis, eIF5A has been suggested to facilitate mRNA nucleocytoplasmic transport and mRNA stability (23, 24). Most recently, the suggested role for eIF5A in mRNA translation has been modified from being involved in translation initiation to also being important for the translation of "hard-to-translate" regions such as polyproline stretches (25; reviewed in references 26 and 27) and translation termination (28).

ROLES OF POLYAMINES IN VIRAL INFECTIONS

Given the abundance of polyamines within the cell and the importance of these molecules for nucleotide charge neutralization, among other functions, it is not entirely

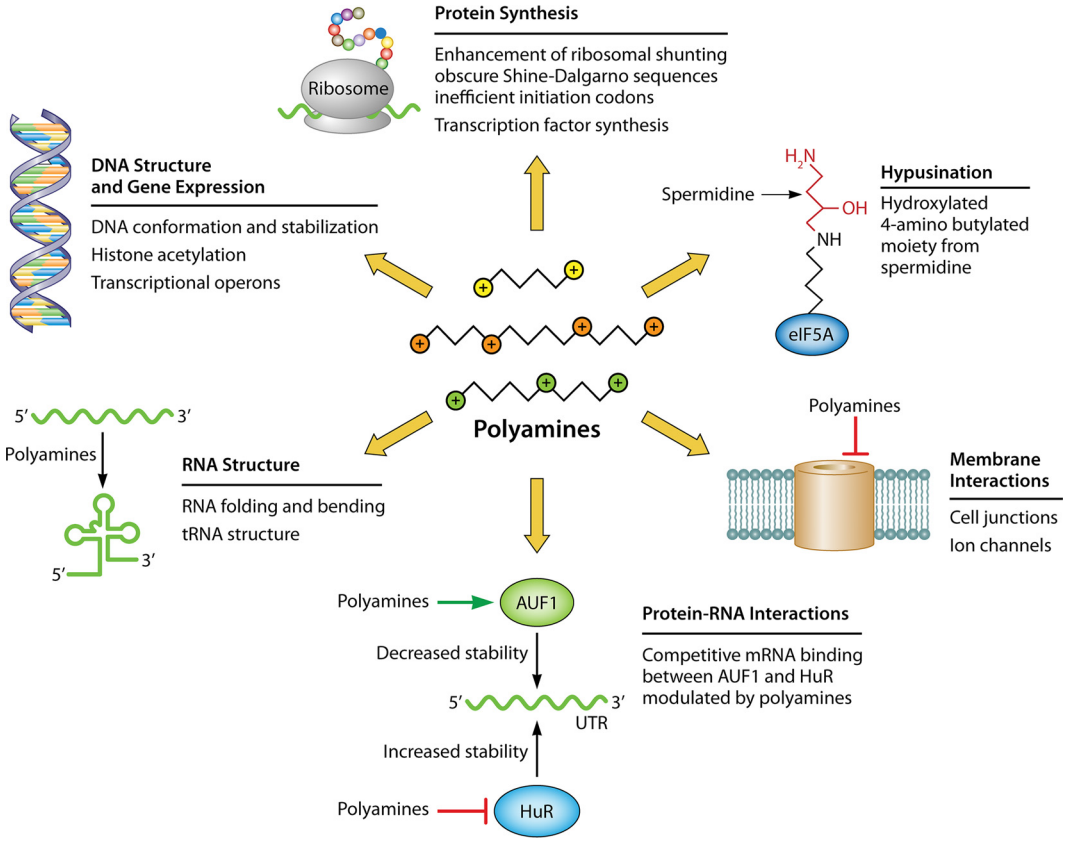

FIG 2 Polyamines in the context of the cell. Polyamines play diverse roles within the cell and alter many of the cellular processes that viruses rely on for their replication, including RNA and DNA structure, protein synthesis and hypusination, membrane interactions, and protein-RNA interactions, as highlighted. AUF1, heteronuclear RNA binding protein D; UTR, untranslated region; HuR, embryonic lethal abnormal vision system human homologue 1.

surprising that viruses utilize and manipulate polyamines for their own replication (summarized in Table 1). Viruses rely on polyamines for numerous stages in the viral life cycle, including genome packaging, DNA-dependent RNA polymerization, genome replication, and viral protein translation. Some viruses also appear to stimulate polyamine synthesis upon infection, highlighting the importance of this pathway for viral replication.

Structural Role of Polyamines in Virions

One aspect of replication where polyamines have been implicated is in packaging the viral genome into virions. Genome packaging is an essential process in the viral life cycle. The negative charges of the DNA/RNA backbone must be balanced if the genome is to be tightly packed. Viruses have developed various mechanisms to facilitate tight packing, including balancing negative charge with a positively charged domain of a capsid protein, surrounding the genome with positively charged single-stranded RNA (ssRNA)/DNA binding proteins, or neutralizing the charge with polyamines (29).

Several DNA viruses utilize polyamines to balance the negatively charged genome within the virion particle. DNA viruses can have large genomes (~190 kb for vaccinia virus and ~236 kb for human cytomegalovirus [HCMV], for example), encoding hundreds of proteins. Several studies have demonstrated that both herpes simplex viruses (HSV) and poxviruses package high concentrations of polyamines, sufficient to neutralize more than 40% of the negative charge on the DNA, depending on the virus (30, 31). In the assembled virion, polyamines are thought to facilitate viral DNA packaging by allowing compaction. Several RNA viruses also encapsidate polyamines but to a lesser extent than DNA viruses, suggesting a less significant role in facilitating packaging

TABLE 1 Roles of polyamines in viral infection

Virus	Virus type	Host factor/ pathway	Proposed mechanism/processes involved	Reagent(s) used	Peak reduction[a]	Reference(s)
Herpes simplex virus 1	dsDNA	Polyamines	Present in virion to neutralize viral DNA			30
			Decreased infectivity through impairment of DNA synthesis	DFMO	2-log	44
			Inhibition of infection; mechanism postentry and synthesis of immediate early genes, before or during DNA replication	MGBG	63-fold	83
Vaccinia virus	dsDNA	Polyamines	Present in virion to neutralize viral DNA	Radiolabeled polyamines		31
			MGBG inhibition late in viral life cycle, viral DNA dissociated from viral inclusions	MGBG	2.9-fold	45
			Upregulation of ODC activity			46
Human cytomegalovirus	dsDNA	Polyamines	Reduction of infectious virus produced; mechanism likely through virus assembly (DNA packaging and/or capsid envelopment)	DFMO	4-log, 6-log	43, 84
Semliki Forest virus	(+)ssRNA	Polyamines	Decreased activity of viral RNA polymerase	DFMO	10-fold	60, 61
Chikungunya virus	(+)ssRNA	Polyamines	Decreased activity of viral RNA polymerase, decreased viral translation, reduction in infectious virus produced	DFMO, DENSpm	200-fold	62, 63
Zika virus	(+)ssRNA	Polyamines	Reduction in infectious virus produced	DFMO, DENSpm	15-fold	62, 63
MERS coronavirus	(+)ssRNA	Polyamines	Reduction in infectious virus produced	DFMO	30-fold	63
Enterovirus A71	(+)ssRNA	Polyamines	Reduction in infectious virus produced	DFMO	12-fold	63
Coxsackievirus B3	(+)ssRNA	Polyamines	Reduction in infectious virus produced	DFMO, DENSpm	10-fold	62, 63
Japanese encephalitis virus	(+)ssRNA	Polyamines	Reduction in infectious virus produced	DFMO	5-fold	63
Yellow fever virus	(+)ssRNA	Polyamines	Reduction in infectious virus produced	DFMO	90-fold	63
Rabies virus	(−)ssRNA	Polyamines	Reduction in infectious virus produced	DFMO	2-fold	63
Rift Valley fever virus	(−)ssRNA	Polyamines	Reduction in infectious virus produced	DFMO	200-fold	63
Vesicular stomatitis virus	(−)ssRNA	Polyamines	Reduction in infectious virus produced	DFMO, DENSpm	20-fold	63
Ebolavirus	(−)ssRNA	Polyamines, eIF5A	Reduction in infectious virus produced, mechanism through decreased accumulation of VP30	CPX, GC7, DEF, DFMO, MDL, SAM486A	3-log	64
Marburgvirus	(−)ssRNA	Polyamines, eIF5A	Reduction in infectious virus produced	CPX	3-log	64
Human immunodeficiency virus	ssRNA-RT	eIF5A	Required for Rev-dependent nuclear transport	eIF5A mutants		67
			Decrease in virus production; required for RNA translation and Rev-induced gene expression	eIF5A siRNA	2-fold	85
			Inhibition of gene expression at transcription initiation	CPX, DEF	200-fold	86

[a]Peak reduction represents experimental time point/condition where the peak effect was observed. Experimental details and alternative time points/conditions can be found in the indicated references.

(32–35). Whether polyamines are enriched in viral capsids as an active process by the virus and, importantly, whether these encapsidated polyamines play roles in addition to packaging have not been fully explored.

Polyamines Stimulate Viral Proteins *In Vitro*

It has been long appreciated that polyamines stimulate activity of viral proteins in *in vitro* assays. Biochemical evidence implicates polyamines in the direct stimulation of purified HSV DNA polymerase (36–38), which falls in line with previous work suggesting that polyamines also stimulate cellular DNA polymerases (39, 40). ORF47, a kinase of varicella-zoster virus (VZV), a betaherpesvirus and the etiological agent of chicken pox, is also stimulated by polyamines (41). Furthermore, the DNA-dependent RNA polymerase of vaccinia virus can also be stimulated in an *in vitro* transcription assay with the addition of spermine or spermidine in combination with Mg^{2+} or Mn^{2+} (42). These results have offered tantalizing hints that polyamines might be important for genome replication in these viruses. These have been followed up to some extent in cell culture-based studies.

Polyamines in the Replication of DNA Viruses

Small-molecule inhibition of cellular polyamine synthesis suggests that polyamines are important for the replication of several DNA viruses. Inhibition of polyamine synthesis with difluoromethylornithine (DFMO) results in a block of both HSV and HCMV replication (43, 44). Additionally, work characterizing the effects of polyamines on vaccinia virus demonstrated that a late step in the viral life cycle, beyond transcription or translation, is affected by methylglyoxal bis(guanylhydrazone) (MGBG), an inhibitor of *S*-adenosylmethionine decarboxylase (SAMDC) (Fig. 1B), which is required for the production of spermidine and spermine (45). Upon MGBG treatment of cells, the association of viral DNA with viral replication factories was reduced. The authors suggested that this phenotype may be a consequence of polyamines being required for maintaining DNA conformation, fitting with this role of polyamines within the cell. Taken together, these studies suggest that polyamines are important in the replication of double-stranded DNA (dsDNA) viruses.

Modulation of polyamine levels by DNA viruses. Virus infection can also result in changes in expression of proteins associated with the polyamine biosynthetic pathway. In the case of vaccinia virus, infected cells exhibit an upregulation of ODC enzyme activity, which is critical in polyamine biosynthesis (46). Vaccinia virus infection significantly inhibits host translation early in infection, and ODC1 is normally an unstable enzyme with a short half-life. This suggests that vaccinia virus may have unique mechanisms to stimulate ODC activity. Human cytomegalovirus (HCMV) similarly stimulates ODC activity within infected cells (47). In addition to vaccinia virus and HCMV, several other viruses also alter polyamine levels and biosynthetic enzymes upon infection, including polyomavirus, adenovirus, and the RNA viruses turnip yellow mosaic virus and hepatitis C virus (48–52), suggesting that many viruses have evolved mechanisms to stimulate the synthesis of the polyamines they need for replication.

In contrast to the case for vaccinia virus and HCMV infection, herpes simplex virus 1 (HSV-1) or HSV-2 infection has been reported to decrease polyamine biosynthesis (53, 54). The differences between HSV and HCMV, which are alpha- and betaherpesviruses, respectively, may reflect distinctions in the infectious cycle for these viruses, such as HCMV's predilection for an S-phase-like cell state or host shutoff by HSV. Whether these changes in polyamine metabolism induced by viral infection affect the replication of either virus is unknown.

DNA virus-encoded polyamine production. While many viruses may alter ODC activity and polyamine metabolism, there is at least one example of a virus encoding its own polyamine metabolism enzymes. *Paramecium bursaria* chlorella virus 1 (PBCV-1), a pathogen of green algae that carries upwards of 700 open reading frames, encodes a complete polyamine biosynthetic pathway (55–58). The presence of these virus genes speaks to the importance of polyamines in PBCV-1 replication. Interestingly,

the product of a gene (Bov2.b2) unique to a bovine gammaherpesvirus (bovine herpesvirus 6) shows sequence similarity to ornithine decarboxylase, with 53 to 56% amino acid sequence identity (59). Again, how this gene functions in the context of infection is unknown.

Polyamines in the Replication of RNA Viruses

The role of polyamines in diverse RNA viruses has also been demonstrated. Semliki Forest virus (SFV), an alphavirus and infrequent human pathogen, was among the first RNA viruses to be studied in polyamine-depleted cells. Treatment of cells with DFMO significantly reduced viral titers, which were then rescued by replenishing polyamines exogenously (32, 60, 61). More recently, a report by Mounce et al. expanded our knowledge of RNA viruses requiring polyamines for replication using DFMO depletion of cellular polyamines (62, 63). The list of RNA viruses sensitive to polyamine depletion has been extended to include diverse families, including alphaviruses (chikungunya virus [CHIKV]), coronaviruses (Middle East respiratory syndrome [MERS] virus), enteroviruses (enterovirus A71 and poliovirus), flaviviruses (dengue virus serotype 1, Japanese encephalitis virus, and yellow fever virus), rhabdoviruses (rabies virus), and bunyaviruses (Rift Valley fever virus) (summarized in Table 1) (62, 63). Replication of each of these viruses was impacted to various degrees when polyamines were depleted with DFMO and rescued when polyamines were replenished exogenously. Furthermore, filoviruses (ebolavirus [EBOV] and marburgvirus [MARV]) were also recently shown to require polyamines through depletion with DFMO treatment (64). Interestingly, these diverse viruses were sensitive to polyamine depletion in several different cell types, including transformed and primary fibroblasts, epithelial cells, neuronal cells, and mosquito cells. This suggests that targeting of polyamines is feasible in a range of cell types which can support various viral infections.

Polyamines in RNA virus transcription and translation. In general, polyamines appear to be important for the midstages of the viral life cycle, e.g., gene expression and genome replication. In SFV infection, polyamines appeared to be necessary for viral RNA-dependent RNA polymerase (RdRP) activity, though it remains unclear whether polymerase activity was decreased due to a lack of polyamines or reduced expression of the polymerase itself (60). Similar to the report with SFV, polyamines also facilitated CHIKV RdRP activity, and further exploration suggested that translation of viral transcripts was also reduced. Similarly, the flaviviruses dengue virus and Zika virus were also sensitive to a depletion of polyamines at the level of translation (62).

eIF5A and Virus Replication

Precisely how translation of viral mRNAs is impacted by polyamines had not been fully understood, but further studies (64) highlighted the unique hypusination of eIF5A as a critical mediator of filovirus protein translation. Perhaps unsurprisingly, given earlier studies suggesting a role for polyamines in the life cycles of several viruses, eIF5A can play an important gating role in virus replication. HIV was the first virus suggested to require eIF5A (94). HIV dependence on eIF5A was reported to occur through Rev-dependent nucleocytoplasmic transport. The HIV-1 Rev transactivator protein is essential for the expression of viral structural proteins and mediates the translocation of viral mRNAs from the nucleus to the cytoplasm (65). eIF5A was shown to specifically bind to Rev (66), and eIF5A loss-of-function mutants blocked the nuclear export of Rev protein and HIV-1 replication (67).

Recent work by Olsen et al. implicates eIF5A in the replication of two additional viruses, ebolavirus (EBOV) and marburgvirus (MARV) (64). In addition to showing that the function of the viral RdRP was strongly decreased in the presence of polyamine inhibitors, the authors showed that this inhibition also occurred when hypusinated eIF5A levels were decreased using various small molecules or when eIF5A was genetically ablated. Treatment of cells with ciclopirox (an inhibitor of DOHH) during infection with EBOV or MARV resulted in a 3-log reduction in infectious titers of these viruses, showing that the inhibition seen using a polymerase activity assay was also observed

TABLE 2 Summary of small molecules which target the polyamine synthesis and downstream hypusination pathways

Molecule[a]	Target[b]	Effect(s) on polyamine levels	Reference(s)
DFMO	ODC1	Inhibition of putrescine synthesis, reduced levels of all polyamines	87
MGBG	SAMDC	Inhibition of spermidine and spermine synthesis	88
SAM486a	SAMDC	Inhibition of spermidine and spermine synthesis	89
MDL-72527	PAOX	Inhibition of polyamine interconversion	90
DENSpm	SAT1	Acetylation of spermidine and spermine, interconversion and removal of polyamines	91
GC7	DHPS	Reduction in eIF5A-deoxyhypusine and -hypusine levels	79
CPX	DOHH	Reduction in eIF5A-hypusine levels	92, 93
DEF	DOHH	Reduction in eIF5A-hypusine levels	92

[a]DFMO, difluoromethylornithine; MGBG, methylglyoxal (bis)guanylhydrazone; SAM486a, sardomozide; DENSpm, N^1,N^{11}-diethylnorspermine; GC7, N^1-guanyl-1,7-diaminoheptane; CPX, ciclopirox; DEF, deferiprone.

[b]ODC1, ornithine decarboxylase 1; SAMDC, *S*-adenosylmethionine decarboxylase; PAOX, polyamine oxidase; SAT1, spermidine/spermine acetyltransferase 1; DHPS, deoxyhypusine synthase; DOHH, deoxyhypusine hydroxylase.

in replicating virus. Further mechanistic probing using the EBOV minigenome system suggested that hypusinated eIF5A is likely required for EBOV mRNA translation.

The requirement of hypusinated eIF5A could offer a potential mechanism for the importance of polyamines in viral replication and will be important to study moving forward. eIF5A has been found to be important in various aspects of protein translation. In addition to relieving stalling at polyproline stretches (25), recent work suggests that eIF5A is important for aiding in the translation of several additional tripeptide motifs as well as translation termination (28), indicating that eIF5A plays a broader role in translation than previously thought. Any one of these roles of eIF5A may provide additional mechanisms for its potential involvement in viral translation.

Polyamines in the Host Response to Viral Infection

The requirement of both polyamines and hypusinated eIF5A for the replication of diverse viruses suggests that cellular control of polyamines could be an effective means of suppressing viral infection. Reducing polyamine levels could restrict the rate or even initiation of virus replication. The interferon response, an innate response triggered by viral infection, detects viral patterns, initiating a series of signaling events that culminate in the expression of interferon-stimulated genes (ISGs) to quell viral infection. Several ISGs directly counteract viral infection by degrading viral RNA, altering membranes, and inducing apoptosis, among many other functions. The polyamine pathway also interfaces with the interferon response; specifically, the spermidine-spermine acetyltransferase SAT1 is upregulated with interferon beta treatment of cells, which results in the depletion of these polyamines and limits viral infection in cell culture (62). Thus, SAT1 acts as a viral restriction factor that limits infection by reducing polyamine levels in cells. Whether SAT1 is upregulated *in vivo* in response to viral infection is not clear.

The Polyamine Pathway as a Therapeutic Target

The polyamine pathway has long been considered a pharmacological target due to the upregulation in biosynthesis in several types of cancer cells. Several molecules have been developed and tested in model organisms to target diverse cancer types (summarized in Table 2 and discussed elsewhere [68]). DFMO, an inhibitor of ODC1, has shown significant promise in the treatment of various cancers in combination therapies (reviewed in reference 69) and is a first-line therapy in the treatment of trypanosomiasis (70). The trypanosomes that infect via the bite of a tsetse fly show sensitivity to polyamine depletion, and DFMO is able to target the trypanosomal ODC1 homolog (71). Thus, DFMO by itself or in combination with other antitrypanosomal drugs has shown efficacy in clearing the parasite. Although large doses of DFMO are required over an extended period of time and must be administered frequently, side effects due to the treatment are relatively mild and reversible (72–74). The success of DFMO in the treatment of trypanosomiasis and cancers highlights a potential therapeutic avenue for targeting the polyamine pathway for other diseases.

Given the breadth of pharmaceuticals targeting the polyamine pathway (Table 2) and their current use, the ability to target viruses with these drugs is a practical strategy. In several animal models, including zebrafish, *Drosophila melanogaster*, and mice, extended pretreatment of the organisms with DFMO resulted in reduced titers of Sindbis virus (SINV), CHIKV, and coxsackievirus B3 (CVB3) (62, 63). However, in the mouse model, the reductions in viral titer in target organs were slight, suggesting that further optimization or a combination therapy may be necessary to effectively quell viral replication.

Another potential therapeutic approach is to target the catabolism of the biogenic polyamines rather than to prevent their synthesis. Treatment of cells with the molecule N^1,N^{11}-diethylnorspermine (DENSpm) results in the upregulation of SAT1 and subsequent acetylation of spermidine and spermine, leading to the reconversion into putrescine or export. DENSpm showed activity against several different viruses in cell culture, including positive-sense RNA viruses (CHIKV and CVB3) and negative-sense RNA viruses (VSV), suggesting that it is effective against diverse RNA virus families (63), and it has been tested in humans, with limited side effects (76). SAM486a, an inhibitor of *S*-adenosylmethionine decarboxylase with antiviral properties against EBOV and MARV (64), has also been tested in clinical trials for non-Hodgkin's lymphoma, with relatively limited toxicity and promising response rates (77). Similarly, the compound *N*,N^1-bis(2,3-butadienyl)-1,4-butanediamine (MDL 72527), which acts by blocking polyamine oxidase and preventing interconversion of the polyamines, was also effective against EBOV and MARV replication in cell culture studies (64), though no clinical trials have investigated its use to date. Given the mechanism of action of these drugs, they may hold promise in combatting viral infection, though further clinical trials would be necessary to explore this avenue.

In addition to targeting polyamines, several molecules have been shown to target the hypusination of eIF5A (reviewed in reference 78). N^1-guanyl-1,7-diamine-heptane (GC7) is a spermidine analog and competitive inhibitor of deoxyhypusine synthase (79). Recent work shows that the use of GC7 is an effective strategy to control EBOV replication in cell culture, suggesting that this compound may be an effective antiviral (64). Also, the compounds ciclopirox (CPX) and deferiprone (DEF), which inhibit deoxyhypusine hydroxylase, have clinical applications as topical antifungal agents, and their antiviral properties in cell culture make them enticing compounds to treat viral infection. CPX was tested in a phase I clinical trial in patients with hematologic malignancies, with limited side effects and promising results (reviewed in reference 80). DEF has also been recently tested in an exploratory trial in treatment-naive HIV-1 patients. Treatment resulted in a marked decline of HIV-1 RNA, without rebound for 8 weeks, well after clearance from circulation (81).

The existence of so many molecules that can be tested for their antiviral properties bodes well for future testing to determine if polyamines and hypusinated eIF5A can be directly targeted to block viral infections (Table 2). Although polyamines and hypusinated eIF5A are important for a number of different cellular processes, there is precedent for targeting these pathways as an antiviral approach. It is important to note that in animal models of viral disease tested so far, DFMO-mediated polyamine depletion was not a panacea. Instead, the effect of DFMO is especially strong in certain organs, including the kidney, liver, and intestines in a mouse model (82). Thus, polyamine depletion may be most effective in these organs and might be used to target viruses such as HCV and EBOV that show tropism for the liver. Additional work will shed light on whether and how these molecules might be useful as viral therapeutics.

CONCLUSION

Despite their small size, the diverse roles of polyamines in cellular and viral processes speak to their importance. Although polyamines were initially characterized in viral studies solely based on their presence or absence in viral capsids, we are beginning to appreciate the additional means by which they facilitate infection of both DNA

and RNA viruses (summarized in Table 1). Much remains to be discovered, however, especially concerning the precise mechanisms whereby polyamines are used in viral replication and how they are involved in immune responses in different organisms. With numerous drugs already developed to target the polyamine pathway that are currently being used in the treatment of other diseases, the possibility to disturb viral infection via the polyamine pathway presents several opportunities that should be explored clinically.

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